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RESEARCH ARTICLE

Efficacy of IAA for affecting nitrate reductase activity and yield attributes of mash [*Vigna Mungo* (L.)] hepper

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ABSTRACT

In order to investigate the potential of Indole Acetic Acid (IAA) for affecting yield contributing factors and Nitrate Reductase Activity, an experiment was conducted on four *Vigna mungo* genotypes. The four mash bean genotypes, 80, 88, 97, and ES-1, were examined against Indole Acetic Acid concentrations of 25, 50, 75, 10, 12, and 150.0 mgL^{-1}. Plants were grown in pots which were randomly placed in the Botanical garden of Bahauddin Zakariya University, Multan. Pakistan. Four replicates of each treatment were developed and evaluated. Aforementioned amounts of Indole Acetic Acid were sprayed thrice, with intervals of ten days each. Ist spray was conducted at twenty days after germination. Nitrate Reductase Activity, grains number $fruit^{-1}$, legumes number $plant^{-1}$, and plant total yield were the traits chosen for study. After ten days of completion of three sprays of Indole Acetic Acid, Nitrate Reductase Activity (NRA) in the leaves was evaluated, and yield $plant^{-1}$ and its contributing factors were noted when the crop reached at its physiological maturity. Typically, an exponential increase in the number of grains, legume per plant, total yield, and Nitrate Reductase Activity (NRA) was seen as Indole Acetic Acid concentration increased from 75.0 to 125.0 mgL^{-1}. By using a statistical technique, it was found that the responses of different genotypes to Indole Acetic Acid concentrations varied. MASH 97 was proved to be the most influenced in term of total yield $plant^{-1}$, the number of grains $plants^{-1}$, and the Nitrate Reductase Activity (NRA). While, MASH ES-1 performance lagged behind all other genotypes.

Keywords: Indole Acetic Acid; Legumes; Nitrate Reductase; Vigna; Yield; Pods

INTRODUCTION

Plant growth regulators are important for the development and regulation of growth. Even at the smallest concentrations, substances that control plant growth can have an impact on a plant's growth and developmental process. Their effects on plants vary according on the type of plant, environmental factors, stage of growth, physiological state, nutrition, and levels of endogenous hormones. Excess exogenous hormones can be stored by plants in conjugate form, where they can then be released in active forms as needed (Davies, 2004).

The hormones known as auxins have the ability to control plant development and growth. Because they are non-toxic over a broad range of doses, auxins are regarded as the best hormones. Auxins control cell lengthening, cell division, callus formation, and loosening of the cell wall (Taiz and Zeiger 2006). Indole-3-Acetic Acid (IAA), which is mostly produced in the apex of shoot and leaf primordia, is the most important auxin. The meristematic cells of flowers, fruits, and immature seeds are additional locations for IAA production (Sadak et al. 2013). The effects of IAA on various crops have been studied by many researchers (Hanaa andSafaa, 2019; Hussain et al. 2020).

Mash bean [*Vigna mungo* (L.) Hepper], crop is among the widely cultivated legume crops (Nag et al., 2006). It contains fats, protein, oil, carbohydrates and traces of vitamins (James, 1981). It is cultivated in Australia, Canada, America and also in Pakistan. This crop is cultivated in Pakistan for the purposes of food, foder and as source of protein in diets. Considering the socio-eonomic importance of crop and effects of Indole Acetic Acid on various crops, this experiment was conducted to find out whether various concentrations of Indole Acetic Acid can express their effects on enhancing yield of Mash crop.

MATERIALS AND METHODS

Indole Acetic Acid (IAA) has been reported to promote the growth, development and production of many crops.

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The goal of the present experiment was to examine the role of Indole Acetic Acid in affecting the number of legumes/plant, number of grains/fruit, total yield/plant(g), and Nitrate Reductase (EC 1.6.6.1) Activity (NRA) in four mash bean genotypes i.e., 80, 88, 97, and ES-1.

Methodology and layout plan

Sandy loam soil that had been dried by the air was filled in plastic containers. The genotypes 80, 88, 97, and ES-1 of mash beans were sown. After germination, three seedlings were kept in each pot by being thinned to ensure a balanced supply of nutrients. After 20 days of germination, first spray of Indole Acetic Acid at concentrations of 25, 50, 75, 100, 125, and 150.0 mg/L (ppm) was applied. For foliar spray the wetting agent used was Tween-20. With ten days interval each, two more sprays were applied. Each genotype and treatment had four replications, and the pots were distributed completely randomized.

Data recordings

Using the Sym, (1984) approach, the Nitrate Reductase Activity in leaves of four plants from each treatment was assessed ten days following the final spray of Indole Acetic Acid. The following chemicals were used to for this purpose.

1) As a stock, 1M solution (156.01 g/l) of NaH_2PO_4 was prepared.
2) Na_2HPO_4 M solution (177.99 g/l) was prepared as the stock solution.

B) Phosphate buffer with a pH of 7.0 and 0.02M KNO_3

1) Sulphanilamide at 1% in 3N HCL
2) 0.02% N(1-Naphthyl-ethylene diamine dihydrogen chloride) (1-Naphthyl-ethylene diamine dihydrogen chloride).

A 20 ml test tube containing 5 ml of media, consisting of 0.01 M phosphate buffer (pH 7.0), 0.02 M KNO_3, and 0.01% Triton x-100, was filled with chopped plant samples (500 mg) of leaf material. For an hour, these test tubes were incubated at 32° C in the dark. After an initial hour of incubation, 1 ml of medium was combined with 0.5 ml of sulphanil amide (10 g/l) in 3 ml of HCl. In order to create pink diazo complex with NO_2, 0.5 ml of N-1-naphthyl ethylene diamine dihydrochloride (0.1 g/l) was added.

After 20 minutes, the dye solution was combined with 5 ml of distilled water and centrifuged for three minutes to remove turbidity. Nitrite analysis was used to test the enzyme. To determine the amount of NO_2 present in the tested media, the standard curve from $NaNO_2$ was drawn. A spectrophotometer reading of 542mm for optical density was made (Hitachi-220). The following formula was used to determine the activity of nitrate reductase:

Nitrate reductase activity was calculated as follows: graph reading dilution factor sample O.D. (mol NO_2/h/g FW).

At crop physiological maturity (90 days age), yield and its allied factors were investigated. Each treatment of every genotype had four plants per treatment that were chosen at random, and counted the number of legumes plant^{-1}, the number of grains fruit^{-1}, and the total yield plant^{-1} (g).

Statistical analysis

Using the COSTAT computer package, data were analysed for Analysis of Variance (two way ANOVA) and the Duncan Multiple Range test was applied with a 5% probability (Duncan, 1955). Using the MSTAT-C Statistical Software, significant F values and LSD values were calculated for comparison of genotypes and treatments.

RESULTS

Number of legumes plant^{-1}

Table 1 contains information regarding the quantity of legumes. When used at concentrations between 75 and 150 mg/L, Indole Acetic Acid significantly increased the number of legumes and had a moderate effect on the frequency of fruit setting. The optimal concentration of Indole Acetic Acid was 125 mg/L, which resulted in a maximum rise of 30.136 percent; the minimum effect (1.412%) was by 25 mg/L. In this regard, 125 mg/L of Indole Acetic Acid was striking in stimulation for legume setting whereas the highest concentration departed from this trend. 50mg/L concentration revealed a reduction in MASH 80 by 4.347% from control.

When recorded individually, response of all the genotypes to this pattern was in a similar fashion. MASH 88 showed maximum (14.047) and MASH 80 showed minimum (12.226) values and rest of the two varieties differed equally from MASH 80.

Number of grains fruit^{-1}

Table 2 contains information on the quantity of grains per fruit. Application of Indole Acetic Acid enhanced the number of grains, and increasing levels of Indole Acetic Acid coincided with a steady increase in the number of grains. The maximum increase (15.020%) was achieved with 125mg/L of Indole Acetic Acid. As for the highest effective Indole Acetic Acid concentration, this was true for all genotypes.

In terms of genotypes differences, MASH ES-1 had the lowest statistical difference (7.530) and MASH 97 had the highest statistical difference (8.340) by IAA applications.

Table 1: Number of legumes plant^{-1} of mash bean exposed to three sprays of IAA concentrations (0, 25, 50, 75, 100, 125 and 150 mg/L) [Means±SE]

IAA (mg/L)	V_1	V_2	V_3	V_4	TREATMENT MEAN (LSD=1.472; n=16)
			(n=4)		
Control	23.000±1.678	25.500±1.568	23.500±3.502	22.000±2.878	23.500^{e}±2.626
25	23.000±1.590 (0.000)	26.164±1.750 (2.603)	24.000±1.964 (2.127)	22.164±1.478 (0.745)	23.832^{de}±2.170 (1.412)
50	22.000±1.440 (-4.347)	27.164±1.988 (6.525)	24.500±1.754 (4.255)	23.164±0.642 (5.290)	24.206^{de}±2.418 (3.004)
75	25.000±3.380 (8.600)	27.334±2.366 (7.192)	24.000±1.632 (2.127)	24.334±2.076 (10.609)	25.166^{cd}±2.570 (7.089)
100	24.500±1.482 (6.521)	30.000±3.568 (17.647)	28.670±1.626 (22.000)	27.164±1.746 (23.500)	27.582^{b}±2.528 (17.370)
125	30.500±3.048 (32.000)	32.164±2.134 (26.133)	30.834±1.370 (31.208)	28.834±1.746 (31.063)	30.582^{a}±2.292 (30.136)
150	23.164±2.390 (0.713)	28.330±1.388 (11.098)	26.834±1.370 (14.187)	28.164±1.666 (28.018)	26.622^{bc}±2.658 (13.285)
GENOTYPE MEAN →	24.452^{c}±3.350	28.094^{a}±2.928 (-14.894)	26.048^{b}±3.194 (-6.527)	23.118^{bc}±3.178 (-2.723)	25.928±3.414
			(LSD=1.112; n=28)		

A value in parentheses represents percentage increase (+)/decrease (-) over control or genotype 80.
Values with dissimilar letters are different significantly (revealed by Duncan Multiple range Test).
V_1=MASH 80, V_2=MASH 88, V_3=MASH 97, V_4=MASH ES-1.

Table 2: Number of grains fruit^{-1} of mash bean exposed to three sprays of IAA concentrations (0, 25, 50, 75, 100, 125 and 150 mg/L) [Means±SE]

IAA (mg/L)	V_1	V_2	V_3	V_4	TREATMENT MEAN (LSD=0.442;n=16)
			(n=4)		
Control	7.657±0.717	7.345±0.421	7.795±1.098	7.015±0.903	7.453^{d}±0.799
25	7.792±0.654 (1.763)	7.045±0.445 (-4.084)	8.252±0.576 (5.862)	7.247±0.416 (3.307)	7.584^{d}±0.682 (1.757)
50	7.492±0.576 (-2.154)	7.267±0.416 (-1.061)	8.082±0.268 (3.681)	7.045±0.445 (0.427)	7.471^{d}±0.560 (0.241)
75	7.757±0.767 (1.305)	8.397±0.721 (14.322)	8.492±0.345 (8.941)	7.587±0.979 (8.153)	8.058^{bc}±0.775 (8.117)
100	8.502±0.668 (11.035)	8.502±0.268 (15.752)	8.760±0.554 (12.379)	8.015±0.963 (14.255)	8.445^{ab}±0.654 (13.310)
125	8.692±0.636 (13.517)	8.502±0.576 (15.752)	8.810±0.334 (13.021)	8.290±0.559 (18.175)	8.573^{a}±0.523 (15.020)
150	8.282±0.520 (8.162)	7.337±0.758 (-0.108)	8.187±0.373 (5.028)	7.515±0.604 (7.127)	7.830^{cd}±0.670 (5.057)
GENOTYPE MEAN →	8.025^{ab}±0.722	7.771^{bc}±0.784 (3.165)	8.340^{a}±0.612 (-3.925)	7.530^{c}±0.790 (6.188)	7.916±0.781
			(LSD=0.334 ;n=28)		

A value in parentheses represents percentage increase (+)/decrease (-) over control or genotype 80.
Values with dissimilar letters are different significantly (revealed by Duncan Multiple range Test).
V_1=MASH 80, V_2=MASH 88, V_3=MASH 97, V_4=MASH ES-1.

Total yield plant^{-1}(g)

Table 3 contains the total yield statistics. Results indicated that yield rose in response to Indole Acetic Acid's stimulations. Indole Acetic Acid statistically had an impact on yield in a concentration-dependent way at concentrations between 75 and 125 mg/L. Mean and individual genotype responses to increasing concentrations of Indole Acetic Acid revealed that 125 mg/L concentration was optimal in this regard. The two lowest and highest concentrations had the most detrimental effects on MASH 80 and MASH 88, causing the yield to change in a slightly declining direction. Genotypes varied in their response to Indole Acetic Acid. MASH ES-1 was the least productive among the genotypes (4.692%), while MASH 97 was the most productive (5.481). MASH 88 was 11.755% more productive than MASH 80.

Nitrate Reductase (EC 1.6.6.1) Activity (µmol NO_2 h^{-1} g^{-1} FW)

After being exposed to Indole Acetic Acid, nitrate reductase activity (NRA) increased exponentially (Table 4). Between 75 and 125 mg/L, there was a clear and statistically significant association between Nitrate Reductase Activity (NRA) growth and rising Indole Acetic

Table 3: Total yield plant^{-1}(g) of mash bean exposed to three sprays of IAA concentrations (0, 25, 50, 75, 100, 125 and 150 mg/L) [Means±SE]

IAA (mg/L)	V_1	V_2	V_3	V_4	TREATMENT MEAN (LSD=0.890;n=16)
			(n=4)		
Control	8.980±1.122	9.530±0.588	9.410±2.328	7.844±1.316	8.940^{d}±1.490
25	8.920±0.380 (-0.668)	9.230±1.116 (-3.147)	9.914±1.284 (5.356)	8.024±0.766 (2.294)	9.022^{cd}±1.102 (0.917)
50	8.204±0.226 (-8.6141)	8.340±1.636 (-12.486)	9.884±0.910 (5.037)	8.154±0.606 (3.952)	9.020^{cd}±1.632 (0.894)
75	9.684±1.874 (7.839)	11.504±1.636 (20.713)	10.190±0.910 (8.289)	8.564±0.606 (9.178)	9.986^{c}±1.632 (11.700)
100	10.594±0.796 (17.973)	13.010±1.730 (36.516)	12.824±1.488 (36.280)	11.070±1.326 (41.126)	11.874^{b}±1.648 (10.447)
125	13.510±2.088 (50.445)	13.954±1.556 (46.421)	13.844±0.698 (47.120)	12.160±0.784 (55.022)	13.366^{a}±1.458 (49.507)
150	8.470±1.748 (-5.679)	9.344±1.714 (-1.951)	10.664±1.316 (13.326)	9.870±0.812 (25.828)	9.586^{cd}±1.534 (7.225)
GENOTYPE MEAN →	9.766^{b}±2.104	10.914^{a}±2.176 (-11.755)	10.962^{a}±1.990 (-12.246)	9.384^{b}±1.798 (3.911)	
			(LSD=0.672; n=28)		

A value in parentheses represents percentage increase (+)/decrease (-) over control or genotype 80.
Values with dissimilar letters are different significantly (revealed by Duncan Multiple range Test).
V_1=MASH 80, V_2=MASH 88, V_3=MASH 97, V_4=MASH ES-1.

Acid concentrations, although lower levels of Indole Acetic Acid could not be statistically justified for their impact. NRA concentration increased by 125 mg/L at its highest point (33.072%), and by 25 mg/L at its lowest point (0.269%). The optimal concentration that had the greatest significant effects on inducing Nitrate Reductase Activity (NRA) in all genotypes was 125mg/L.Applying lower quantities of Indole Acetic Acid in MASH 80 and MASH 97 resulted in a small increase in nitrate reductase activity (NRA).

Among the genotypes, 97 expressed the highest value (9.612) while ES-1 displayed the lowest (8.897). MASH 97 was valued at 5.300% less than MASH 80.

DISCUSSION

Results of the current experiment showed that Indole Acetic Acid increased the quantity of grains, legumes, and overall plant production (Table 1-3). Duration of leaf area during grain growth also factors for changes in the yield (Wolfe et al., 1988). The formation and development of flower, fruit, and seed are all aided by plant growth chemicals, which can also improve photoassimilates translocation to sink. This increases crop yield (Ammanullah et al. 2010). Significant impacts of IAA on plant grain yield were reported by Gherroucha et al. in 2011. IAA's ability to promote concentration and the transfer of assimilates from leaves to fruits may account for its influence on increasing seed weight (Mostafa et al. 2005; Chen et al. 2014; Mekki 2016; and Kumar et al. 2018).

The research reports indicate that Indole Acetic Acid is beneficial for plant growth and development. Singh et al. (2015) noted, IAA as stimulator that is triggered by tryptophan and is essential for the formation of the strongest plant growth characteristics. One of the most well researched hormones involved in plant growth and development is Indole Acetic Acid, which is produced via both tryptophan (Trp)-dependent and Trp-independent processes from indole-3-acetic acid (Korasick et al., 2014).

It was shown that the use of IAA considerably improved the morphological, physiological, yield, and biochemical characteristics (Hussain et al., 2020). The fact that auxin and gibberellins (GA) created a considerable stimulation for plant development may be the cause of the rise in morphological characteristics. Plant growth regulators were found to have a significant favourable impact on the soybean yield characteristics at various doses. The greatest number of green pods, pod length, and pod weight were observed with IAA at higher concentrations (150 ppm). Together, GA3 and IAA application demonstrated better accumulation of net photosynthetic rate and produced more C: N ratio, which facilitated the growth of the plants (Sudadi and Suryono, 2015). The involvement of IAA in promoting a variety of processes, including as cell division and tissue growth, phototropism and gravitropism, apical dominance, lateral root initiation, differentiation of vascular tissues, embryogenesis, senescence, and ripening, in plants to increase growth and development (Naeem et al., 2004). Application of 100 ppm IAA at the booting stage had a strong positive impact on the yield

Table 4: Nitrate Reductase (EC 1.6.6.1) Activity (NRA) of mash bean exposed to three sprays of IAA concentrations (0, 25, 50, 75, 100, 125 and 150 mg/L) [Means±SE]

IAA (mg/L)	V_1	V_2	V_3	V_4	TREATMENT MEAN (LSD=0.424;n=16)
			(n=4)		
Control	8.59±0.556	8.787±1.129	8.822±0.300	7.992±0.631	8.548^{d}±0.730
25	8.54±0.166 (-0.582)	9.017±0.638 (2.617)	8.627±0.578 (-2.210)	8.102±0.388 (1.376)	8.571^{d}±0.545 (0.269)
50	8.175±0.097 (-4.831)	8.952±0.233 (1.877)	9.045±0.170 (2.527)	8.152±0.354 (2.002)	8.581^{d}±0.480 (0.386)
75	8.91±0.881 (3.728)	9.212±0.428 (4.836)	9.767±0.778 (10.711)	8.33±0.285 (4.229)	9.055^{c}±0.785 (5.931)
100	9.35±0.392 (8.847)	10.51±0.730 (19.608)	10.562±0.750 (19.723)	9.585±0.638 (19.932)	10.001^{b}±0.802 (16.998)
125	11.6±0.602 (35.040)	11.33±0.673 (28.940)	11.472±0.543 (30.038)	11.1±0.386 (38.888)	11.375^{a}±0.537 (33.072)
150	8.492±0.860 (-1.140)	9.41±0.656 (7.090)	8.99±1.049 (1.904)	9.017±0.393 (12.825)	8.977^{cd}±0.773 (5.018)
GENOTYPE MEAN →	9.093^{b}±1.213	9.602^{a}±1.083 (-5.300)	9.612^{a}±1.151 (-5.707)	8.897^{b}±1.139 (2.155)	
			(LSD=0.320; n=28)		

A value in parentheses represents percentage increase (+)/decrease (-) over control or genotype 80.
Values with dissimilar letters are different significantly (revealed by Duncan Multiple range Test).
V_1=MASH 80, V_2=MASH 88, V_3=MASH 97, V_4=MASH ES-1.

attributes and improved wheat grain yield (Hanaa and Safaa, 2019).

In plant cells, an enzyme called NR turns nitrate into nitrite. Previous demonstrations have indicated that exogenous IAA improves NR activity (Hayat et al. 2006; Ali et al. 2008; Ahmad et al. 2001). Auxin and its isoforms affect the activity of NR (Ahmad and Hayat 1999).

Because its high external supply promotes a reduction in endogenous IAA level by a feedback regulatory mechanism, IAA exhibits its declining effects on development at high concentrations (Ahmed et al., 2001). (Ribnicky et al. 1996).

CONCLUSIONS

The results obtained from the present experiment indicated that Indole Acetic Acid @ 125 mgL^{-1} concentration was the most effective and might be a used practice for commercial production of mash. However, this should be economically tested under different conditions of environment and for different cultivars.

CONFLICT OF INTEREST

Authors have no conflict of interest.

Authors Contribution

Ghulam Yasin designed and conducted experiment. Adeela Altaf and Ikram ul Haq participated in writing of manuscript and proof reading.

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